

USSN 09/975,139

**LIST OF CLAIMS, SHOWING THE STATUS OF EACH CLAIM:**

Underlining denotes added text while strikethrough denotes deleted text.

1. (Currently Amended) A method of creating a library of DNA sequences, said method comprising:

a) providing a DNA sequence that encodes a protein of interest;

b) providing a probability matrix for the protein;

c) providing a constraint vector for the protein;

d) applying the constraint vector to the probability matrix to produce a substitution scheme recommending substitutions at least two residues in the protein; and

e) creating a library of DNA sequences incorporating changes in the DNA sequence that produce the recommended substitutions, wherein said library is produced in a bacterial host.

2. (Original) The method of claim 1, wherein said protein is selected from the group consisting of an esterase, dehydrogenase and hydrolase.

3. (Original) The method of claim 2, wherein said protein is selected from the group consisting of a protease, cellulase, lipase, hemicellulase, laccase, and amylase.

4. (Original) The method of claim 1, wherein said protein is selected from the group consisting of a transcription factor, growth factor, antibody, interleukin, antigen, and receptor.

5. (Original) The method of claim 1, wherein the probability matrix is based on structural characteristics selected from the group consisting of conservative residues, sequence alignments, three dimensional structure, residue environment, solvent accessibility, residue chemistry, propensity for a particular secondary structure, and combinations thereof.

6. (Original) The method of claim 1, wherein the constraint vector is based on structural characteristics known to affect protein function selected from the group

USSN 09/975,139

consisting of proximity to the site of functionality, distance of  $\alpha$  or  $\beta$  carbons, contact with residues of interest, and contact with residues that contact the residue of interest.

7. (Cancelled)

8. (Cancelled)

9. (Cancelled)

10. (Cancelled)

11. (Currently Amended) A method for creating at least one library of nucleic acid sequences that encode variants of a protein, said method comprising the steps of:

a) identifying an initial nucleic acid sequence that encodes a desired protein;  
b) establishing a probability matrix for said protein, wherein said probability matrix is generated by a computer; and  
c) generating a constraint vector for said protein, wherein said constraint vector is generated by a computer; and  
d) applying said constraint vector to said probability matrix to produce a substitution scheme; and

e) creating a library of DNA sequences, wherein said library is constructed in a bacterial host.

12. (Cancelled)

13. (Cancelled)

14. (Original) The method of claim 1, wherein a library comprising at least 25 unique DNA sequences is produced.

15. (Original) The method of claim 14, wherein a library comprising at least 100 unique DNA sequences is produced.

USSN 09/975,139

16. (Original) The method of claim 15, wherein a library comprising at least 250 unique DNA sequences is produced.

17. (Original) The method of claim 16, wherein a library comprising at least 1000 unique DNA sequences is produced.

18. (Original) The method of claim 17, wherein a library comprising at least 2500 unique DNA sequences is produced.

19. (Original) The method of claim 18, wherein a library comprising at least 10,000 unique DNA sequences is produced.

20. (Original) The method of claim 1, wherein a library of less than  $10^9$  unique DNA sequences is produced.

21. (Original) The method of claim 20, wherein a library of less than  $10^6$  unique DNA sequences is produced.

22. (Original) The method of claim 21, wherein a library of less than  $10^5$  unique DNA sequences is produced

23. (Previously Presented) The method of claim 1, wherein the probability matrix is generated using an algorithm.

24. (Cancelled)

25. (Previously Presented) The method of claim 1, wherein the constraint vector is generated using an algorithm.

26. (Original) The method of claim 1, wherein the constraint vector is generated by a computer.

27. (Original) The method of claim 1, wherein the constraint vector is

USSN 09/975,139

applied to the probability matrix using a computer.

28. (Original) The method of claim 1, wherein the probability matrix is normalized.

29. (Original) The method of claim 1, wherein the DNA sequence is generated from DNA shuffling.

30. (Cancelled)

31. (Currently Amended) A method of creating a library of DNA sequences, said method comprising:

a) providing a substitution scheme produced by applying a constraint vector to a probability matrix wherein the substitution scheme recommends substitutions at least two residues in a protein of interest; and

b) creating a library of DNA sequences incorporating substitutions in a DNA sequence encoding the protein of interest to create a library comprising the recommended substitutions, wherein said library is created in a bacterial host.